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DEPARTMENT OF THE ARMY
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Study of Cultural and Biochemical Characteristics
of Bacilli of the type
"Hemophilus Hemolyticus vaginalis"

Their sensibility to antibiotics

by

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In the course of former studies on leucorrhea we have noted the frequent presence of innumerable small Gram-negative bacilli found on direct examination of vaginal smears (1,2,3). Recently, we reported briefly the hemophilic and glucidophilic properties of these germs.

In a work parallel to ours, Gardner and his collaborators (5) have reported on a clinical study with some bacteriological data on a bacterial vaginitis due to an organism of the genus *Hemophilus*.

Since the beginning of our research in 1953, we have stated that these small Gram-negative germs could be found in leucorrhea in an almost pure state, although associated with white staphylococci, pseudo-diphtheria bacilli, various streptococci, enterococci, colibacilli, bacilli of Doederlein and Trichomonas. In several cases we have found them alongside of Trichomonas and fermentation fungi of the *Candida* type.

In this study we present the results of our research on their characteristics of culture, their biochemical properties and their sensibility to antibiotics.

Data Furnished by Direct Examination

In all cases where these small bacilli are found in nearly pure state, we have been struck by the absence or rarity of leucocytes. One finds by count a sufficient number of epithelial cells (fig. 1 and 2).

The shape of these germs is nearly that of Pfeiffer bacillus in culture (fig. 5). They measure from 0,9 to 2 μ in length and from 0,3 to 0,5 μ in width. More or less polymorphic, they appear in the form of bacilli, coccobacilli, diplobacilli and more rarely of diplococci or cocci (fig. 3,4).

These small Gram-negative bacilli are immobile, non-sporegenic. We have not seen evidence of capsule. They are easily stained with methylene blue, phenolized fuchsia and phenolized thionine. Certain elements are intensely colored.

Cultures

On solid media. — After a check of our cultures on usual media (agar, blood agar, ascetic fluid, etc.) and on certain special media like that of Bordet-Gengou, gonococcal media, media for P.P.L.O., Difco with serum or ascetic fluid, we proceeded with different experiments to determine the factors necessary for growth. The results of our attempts are outlined in the table below, summarizing results obtained on ten strains.

As this table shows, the best results were obtained in 48 hours on media containing blood (10%), glucose and extract of yeast. These media should be fresh, newly prepared. Neither the atmosphere of CO_2 nor the presence of staphylococci (factor V) have favored growth. Sheep blood has been more favorable than rabbit blood.

Here are the Cultural Characteristics of the Two Media. — Medium I (nutritive broth and blood 10% + glucose 3 % + yeast extract). — Forty-eight hours after seeding tiny colonies appear which grow larger during the following days. The surface of colonies is smooth; the borders regular. Colonies are surrounded by a greenish halo with a thin border of hemolysis at its edge.

Medium II (base of proteose peptone Difco). — Colonies appearing after 48 hours are surrounded by a zone of hemolysis (fig. 6). From the morphological point of view coccoidal forms are most often seen in culture (fig. 7,8) and sometimes bacilli more or less long (fig. 9). In addition, the morphology may vary and after numerous transplants of coccoidal forms, the germs may take the form of bacilli (fig. 9).

The viability of primocultures is 3 to 5 days.

On Liquid Media. --- In starting cultures on solid media our research has lead, after transplants, to the results summarized in table II.

This table shows the necessity of adding blood and glucose. Amounts of glucose varying from 1 to 5 p. 1,000 have not made any appreciable difference for the cultures.

On liquid medium, after 48 hours for the first transplant and 24 hours for the following, there should appear a light troubling. On medium I, one observes sometimes first a slight deposit on the side of tubes followed by the appearance of a uniform troubling. After a few days, the red globules are more or less strongly hemolyzed. The morphology of germs in liquid media. (fig. 10) resembles that described on solid medium. Viability of these germs in liquid medium lasts 8 days and by successive transplants the strains can be stretched out for several weeks.

Biochemical Characteristics

Our research on the use of sugars has given the following results

(See Table III)

In any case, we have not been able to show production of indol nor reduction of nitrates.

Experimental Pathogenic Capacity

Mice inoculated by intraperitoneal route have not presented any trouble and have survived. Intradermal injections of strains to rabbits have not occasioned necrosis.

Sensibility to Antibiotics

Following are the results obtained by the method of discs for 10 strains studied. (See Table IV)

CONCLUSION

This work shows us that the small Gram-negative bacilli frequently found in vaginal smears and capable of causing leucorrhea formerly considered non specific, are bacilli of the genre *Hemophilus*. They are glucidophilic and possess hemolytic properties more or less accentuated according to the medium of culture, which facilitates their diagnosis.